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BALLISTIC DELIVERY OF BIOLOGICAL REAGENTS

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ABSTRACT

This study was undertaken to demonstrate the feasibility of delivering biological reagents at useful ranges (up to 40 meters) using conventional small arms weapons. The primary application for the technique is in the control of infectious diseases among unrestrained animals. Three drug preparations are considered: a tranquilizing or restraining preparation for swine, an attenuated Venezulean Equine Encephalitis vaccine for horses, and a terminal anesthetic for use in hog cholera depopulation procedures.

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BALLISTIC DELIVERY OF BIOLOGICAL REAGENTS

I. INTRODUCTION

For the past five years this laboratory has been active in developing an improved method of restraining wild animals.⁽¹⁾ This report covers the eight-month period from November 1972 to July 1973, during which time special consideration was given to problems of disease control in domestic animals.

Several manufacturers produce capture guns that deliver biologic materials to unrestrained animals. These operate by injecting a chemical restraint agent upon impact.* The most common technique is to construct a projectile in the form of a metal syringe that carries an active drug in solution. Our field experience with this technique led to the current work in order to first, increase the ballistic accuracy with which drugs could be delivered (we consider a 2-inch diameter, or 5 cm, target at 100 yards a reasonable and attainable standard); and second, to reduce the physical injury caused by forceful injection of the liquid solution. As an example,

*Palmer Chemical Company, PaxArms, Pseudart

the Palmer "CapChur Charge" is an inertia activated explosive used to inject the solution after impact of the syringe. While the secondary explosive is a reliable method of forcing the syringe plunger to operate, it is our opinion that considerable unnecessary trauma is produced by this injection method. Ideally, the drug should penetrate a muscle only superficially.

The compacting of dry active drugs into a small .22 caliber projectile offers numerous conceptual advantages over commercially available methods:

1. Ballistic accuracy
2. Standard doses
3. Generally better storage life is expected for drugs in dry form than those in solution.
4. Ease and speed in handling active materials in the field.
5. Approximately the same diameter surface penetration as the syringe dart barbed needle.
6. Approximately the same reaction time for soluble materials as in the liquid form.
7. Superficial muscle penetration is obtained using high velocity projectiles with low mass or inertia.

Some new problems arise, however, with solid drug projectiles. For example, restraining drugs with wide dosage latitude are desirable but scarce. In addition, a projectile hard enough to be fired without shattering must be made to dissolve rapidly in body fluids, and it must therefore be protected from moisture prior to use. Nevertheless, the advantages of the pellet projectile

have been realized to a great degree, and the inherent problems overcome in work prior to this contract.

In 1971-72 an outbreak of hog cholera occurred in the central and eastern United States. Control of this disease is routinely effected by destroying all swine where the disease is found to exist, and compensating the owners for their losses. This method of depopulation generally involves physical restraint of swine and injection with succinylcholine chloride to produce apnea and suffocation by blocking muscular nerve action. The need to physically restrain large boars and sows to administer the succinylcholine is time consuming and occasionally results in injuries to disease control personnel. There is also considerable risk to persons in handling succinylcholine in solution because it is absorbed through the skin.

As a result of the foregoing, this project was initiated to determine--

- (a) if a tranquilizing material could be delivered using a pellet projectile at short range which would reduce the trauma associated with physical restraint of swine; and
- (b) if a terminal anesthesia could be developed for pellet delivery for use in rapid depopulation to

eliminate the problems of administering succinylcholine chloride in solution.

A second objective of this project was to demonstrate the feasibility of administering vaccines to unrestrained animals using projectiles containing live attenuated virus when disease control is effected by vaccination. In such procedures all susceptible animals in a given region should be inoculated in order to insure that unvaccinated animals do not serve as reservoirs for the spread of infection in the future. Venezuelan Equine Encephalitis (VEE) was selected to test the effectiveness of vaccine delivery by projectiles. While domestic horses can be vaccinated using standard methods, many hundreds of feral horses and donkeys cannot be captured and treated without great expense. To demonstrate an alternate solution to this problem, efforts were directed toward--

- (1) development of a medium-range inert projectile to act as a carrier for vaccines; and
- (2) the development of a short-range projectile for conducting a test of vaccine effectiveness after ballistic delivery.

A cooperative program with the National Animal Disease Laboratory (NADL), Ames, Iowa, was established

to verify the effectiveness of vaccines delivered in a solid pellet form. Dr. James Pearson, Virologist, was responsible for this phase of the investigation.

II. HOG CHOLERA CONTROL METHODS

A. Chemical Restraint Projectiles

Development of a chemical restraint pellet was based on several assumptions:

- (a) that delivery would be at close range to penned animals;
- (b) that reaction to the drug would need to be rapid; and
- (c) that drug selection should be based on humane considerations.

In view of the close range of delivery, a .22 caliber compressed air rifle was selected for administering the pellets. A number of manufacturers produce inexpensive air rifles suitable for this purpose. The Crossman Model 1400, and the Smith and Wesson Model 77-A, were used in tests involving pellet delivery. Both guns are single shot, manual loading and provide a hand-operated pump to develop propulsion energy. Between 8 and 20 pump actions were used for each round, depending on range.

Using 8 pump actions and standard commercial lead pellets ("Superpells"), the penetration into a pine board at 21 feet was between 1/32" and 2/32" for each rifle.

B. Drug Selection and Formulation

A large number of chemical restraint drugs had been tested in previous work with wild animals in a study conducted for the Arizona Game and Fish Department. In this work the use of Tiletamine (Parke-Davis CL634)^{(2),(3),(4)} was indicated because of rapid induction time, relative potency and latitude. The drug produces a state of amnesia, or ataractic condition, in which breathing and heart action are not adversely affected. The following active formulation was prepared:

Tiletamine	96 mg
Acepromazine	14 mg
Hyoscine	3 mg
Hyaluronidase	6 mg

Finely powdered materials were pressed in a Stokes Model B tablet press with suitable inert binders to produce a hard pellet. Tablets were found to dissolve completely in agitated water in less than 20 seconds.

C. Ballistic Tests

A large number of rounds were fired at various ranges to determine accuracy and penetration. Typical accuracies at 21 feet with 20 pump actions using the Smith and Wesson 77-A were such as to place all rounds in a circle about one inch in diameter.

Penetration was determined by using "Duxseal," a commercial clay material used in sealing air conditioning

ducts. Penetration in Duxseal was found to approximate penetration in muscle mass. Typical penetrations were about 5/16" using 15 pump actions at 15 feet, and 20 pump actions at 21 feet.

D. Test Results

A 70-pound male pig was used to confirm drug selection and delivery feasibility. The pellet was delivered to the right rear leg at about 25 feet range using 20 pumps with the Smith and Wesson 77-A air rifle.

The pig assumed a recumbent position in 6-1/2 minutes. The animal could be aroused to an ataxic standing position for 28 minutes after delivery, but thereafter was generally immobile. Recovery was gradual and the animal was able to stand within two hours after pellet administration.

E. Conclusion

The effectiveness of the Tiletamine combination was expected from previous work with canines (wolves and coyotes), javelina and deer. The particular form of amnesia produced by Tiletamine is thought to be a humane form of restraint and should be considered as a preparative agent prior to the use of muscle blocking agents for eradication of diseased animals. At the time

Tiletamine was used it was considered an experimental drug by the manufacturer and was not available through veterinary supply channels. This precludes consideration of cost and availability factors in recommending its use.

III. DEPOPULATION TECHNIQUES

A. Restraint Weapon

The pellet air rifle was again selected for application in this study.

B. Drug Selection and Formulation

The primary objective for this phase of the investigation was selection of a rapid acting, potent and inexpensive death-producing agent. Several other considerations were involved, however: The pellets could be lost during depopulation procedures and possibly be eaten by farm animals or children. Succinylcholine chloride had previously been used in wild animal restraint applications, but the largest air rifle projectile using this material (88 mg) was found to be an insufficient dose for 70-pound swine.

After consideration of a number of biological agents, it was decided to select a more potent muscle blocking agent, such as Dimethyl Tubocurarine Iodide (DMTC). The LD-50 for Swiss Webster mice was determined to be 0.38 mg/kg with the following formulation:

DMTC	3%
Acepromazine	32%
Hyoscine	4%

Chemical restraint with recovery occurred between 0.237 and 0.353 mg/kg with no residual effects. Four young pigs weighing between 27 and 35 pounds were used to establish the potency of the DMTC combination in solution, administered intramuscularly. As a result of a series of tests with these animals, a final tablet was formulated using:

DMTC	25%
Acepromazine	10%
Hyoscine	3%
Hyaluronidase	2%
inert binder	60%

Each tablet contained 12.5 mg of DMTC and was estimated to produce death in less than 8 minutes in swine weighing approximately 70 pounds. Up to 5 separate tablets may be combined to fabricate a pellet projectile. Pellets dissolved in agitated water in approximately 8 seconds.

C. Ballistic Tests

Ballistic accuracy and penetration were found to be similar to the chemical restraint pellets fabricated earlier.

D. Test Results

Approximately 50 rounds were prepared for testing

under field conditions. Unfortunately for this phase of the study, an opportunity for evaluation was not available due to the decrease in incidence of hog cholera.

IV. VEE VACCINE DELIVERY

A. Medium-Range Inert Projectile

Development of an accurate round for a conventional rifle is an art. It requires a delicate balancing among a large number of variables, which include as major factors--powder burning rate, barrel twist, length of barrel, and mass of the projectile. Very low mass projectiles suitable for carrying biologics into the body of an animal with only superficial injury require high rotational velocities for ballistic stabilization. The smallest diameter round sufficient to carry the active material is desirable to reduce wound size. The .22 caliber weapons were selected with these factors in mind. The common inexpensive rim fire cartridge may be the most desirable for use ultimately, but center fire cartridges offer more flexibility for experimental use in the development of new projectiles. Development of a medium-range vaccine projectile was based on the .223 caliber center fire cartridge shown in the photograph following (Figure 1).

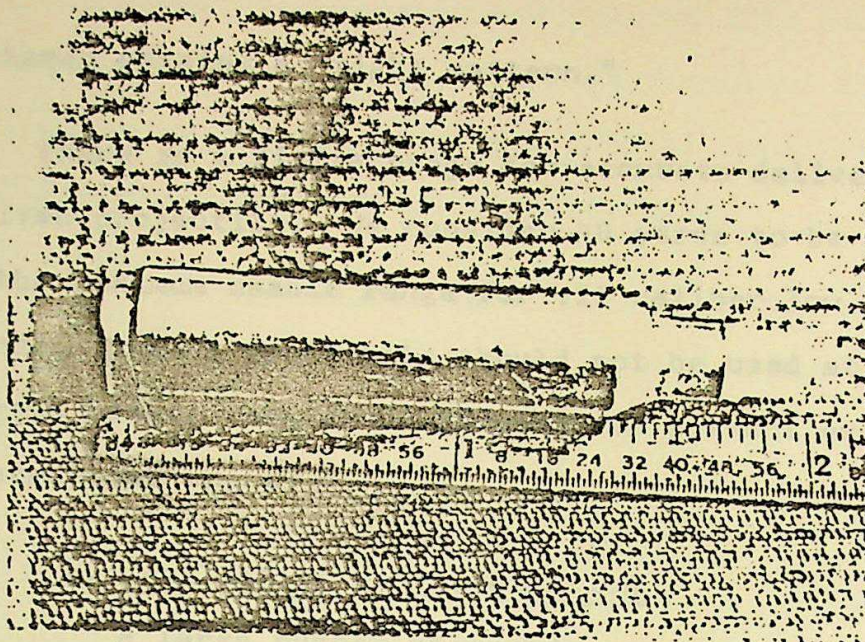


Figure 1.

It was expedient to consider projectile specification in two parts: an active vaccine tip and an inert plastic body or carrier (sabot). The function of the plastic body is to conform to the inner surface of the rifle barrel and form a seal to contain the propulsive gas pressure while the round is travelling through the barrel. The spiral ridges in the barrel impart spin to the projectile during this time, which is necessary for stabilization. The tip of the projectile must be shaped properly for penetration of the air, and body surface and longitudinal balance must be correct to prevent precession or wobbling during flight. When all of these factors are properly considered a good

marksman obtains a "tight pattern."

There is a finite range for accurate delivery of a given projectile. We consider 40 yards to be close to the maximum usable range for .22 caliber low mass rounds. Such a projectile should not be used at live targets closer than about 25 yards to prevent excessive penetration.

1. Ballistics and Penetration

A large number of rounds were constructed and fired. Bullseye pistol powder, a rapid-burning explosive, was used for propulsion. Typical results were as follows:

<u>Powder (Grains)</u>	<u>Range (Yards)</u>	<u>No. Rounds</u>	<u>Pattern (Inches)</u>	<u>Duxseal Penetration (Inches)</u>
4	30	11	1.9	.25 to .32
4.5	30	6	0.7	.3
5	40	3	2.3	.4
5.5	40	3	1.6	.4
5.5	35	2	1.2	.4

2. Vaccine Formulation

VEE vaccine was obtained either through veterinary supply channels or direct from the manufacturer--Jensen-Salsbery Laboratories, Kansas City, Missouri. Virus was stored under vacuum at -11°C when not being used in pellet fabrication.

Dry vaccine was mixed with Avicel 102 (Food Machinery Corporation) and pressed into hard tablets using a Stokes press. Tablets dissolved in agitated water in 8 to 19 seconds. Initial formulation was 3 parts vaccine to 14 parts Avicel.

Vaccine tablets were fastened to Delrin (polycarbonate) bodies with Duco Cement. Pellets contained 1.5 normal doses of vaccine after being rounded to form the projectile tip. Figure 2 shows projectiles recovered after firing into Duxseal.

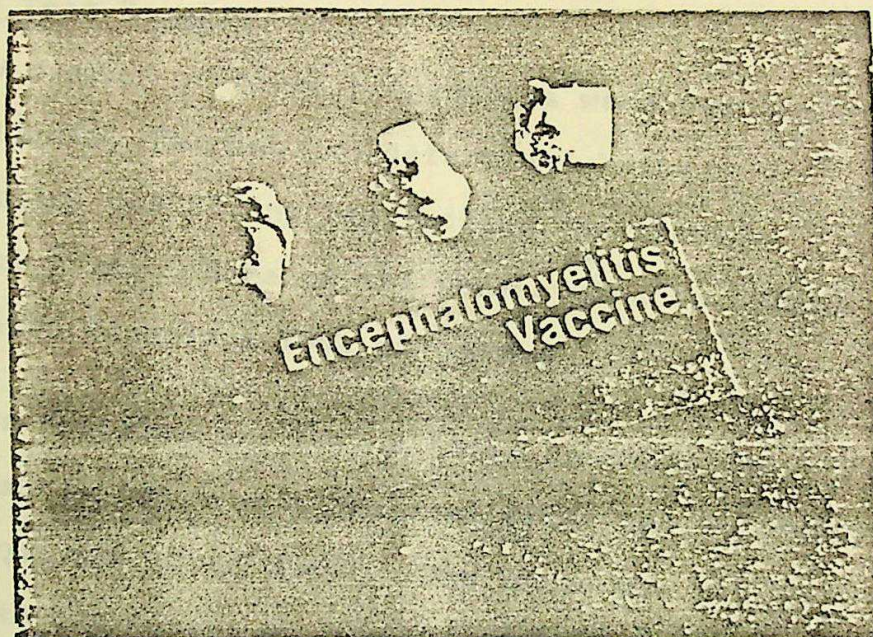


Figure 2.

3. Dye-Marking Inoculated Animals

A permanent but non-injurious dye, gentian violet, was encapsulated in a gelatin base and tested for its effectiveness in surface marking animals without penetration of the hide. The dye-carrying projectile performed well in tests and is considered to be a useful addition to the drug pellet system. Combined with therapeutic or restraining drugs the dye marking technique provides a convenient means for identifying animals that have been inoculated.

4. Test Results

A number of fired projectiles were furnished to NADL (Dr. Pearson) to determine whether processing and firing had reduced the vaccine effectiveness. None of the projectiles so tested indicated a serious loss of potency.

On November 17, 1972 a horse belonging to Charles Williams was inoculated using the delivery technique described above. The horse was hit in the rear leg muscle at 30 yards range. Slight swelling developed initially but the surface wound and swelling disappeared within 5 days. No lameness was noted.

B. Short-Range VEE Pellet

On the basis of encouraging results with the medium-range projectile, it was decided to proceed with a full scale statistically significant test at NADL involving 18 ponies. Because some concern was expressed regarding the possibility of contamination with lost vaccine pellets, it was agreed to conduct the test at short range in the building where the horses were stabled. A .22 caliber air rifle pellet was developed and tested for this purpose.

1. Preliminary Testing

Preliminary testing of fired pellets indicated a slight decremental loss of effectiveness. As a result, the ratio of vaccine to Avicel was increased to 1:6.77 and the projectiles were increased slightly in size to deliver 1.7 equine doses per tablet. A rounded (semispherical) tip was made using $\text{Ca}_3(\text{PO}_4)_2$ to aid in penetration of hide and to avoid shaping the active vaccine tablet.

A test of the air gun pellet was conducted with a horse on March 27, 1973. Good penetration was obtained using 8 pump actions with the Smith and Wesson 77-A at 6-yard range. Healing was again complete in 5 days.

2. Final Testing

Eighteen ponies were vaccinated at NADL on 1 May 1973. Eight to 12 pump actions were used with the Smith and Wesson 77-A at ranges of 8 to 12 feet. All 18 animals developed titer to VEE. Pellet wounds healed without medication. Some temporary drainage was noted in 9 of the animals. A detailed report of this experiment is being prepared by Dr. Pearson.

V. CONCLUSION

The feasibility of delivery of various biological agents for disease control has been explored and found to be affirmative. Additional field testing of both restraining and depopulation techniques for application to hog cholera control is necessary before a field procedure can be recommended. There seems to be no major technical obstacle to overcome in this application, however.

The concept of administering vaccines to unrestrained animals has been found to be possible and effective. A detailed report of this procedure is being prepared for publication.

ACKNOWLEDGMENTS

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